



Effect of calcium salts of fish and palm oils on lactational performance of Holstein cows

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Abstract

Two experiments were conducted to determine the effect of source of fatty acids (FA) fed as tallow or calcium salts of fish and palm oils (CaSFO) on performance of dairy cows. In experiment 1, 738 multiparous Holstein cows were fed a diet with either 18 g/kg of tallow or 19 g/kg of CaSFO to supply equal amounts of FA in two replicates per treatment. In experiment 2, 331 multiparous cows were fed one of the two supplements described previously. In both experiments, all cows received a pre-treatment diet during the first 25 days in milk (DIM) consisting of half of each fat supplement (9 g/kg of tallow + 9.5 g/kg of CaSFO). Thereafter, cows received their respective diets for the first 145 DIM. Yields of milk and milk fat were not affected by dietary source of FA in both experiments. However, feeding CaSFO reduced concentration of true protein and solids-not-fat, as well as yields of true protein. Group and individual dry matter (DM) intakes and feed efficiency were not altered by treatment, but cows fed CaSFO had higher total tract apparent digestibility of DM. Cows fed

Abbreviations: ADF, acid detergent fiber; CaS, calcium salts; CaSFO, calcium salt of fish and palm oils; CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; DIM, days in milk; DM, dry matter; EPA, eicosapentaenoic acid; EXP-1, experiment 1; EXP-2, experiment 2; FA, fatty acids; NEL, net energy of lactation; NDF, neutral detergent fiber; PUFA, polyunsaturated fatty acids; RUP, ruminally undegradable protein; SCC, somatic cell count; TMR, total mixed ration; UFA, unsaturated fatty acids

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CaSFO had increased concentrations of eicosapentaenoic, docosahexaenoic, conjugated linoleic acid (*cis* 9, *trans* 11), C18:1 *trans* 9, and C18:1 *trans* 11 FA in milk fat. Treatment had no effect on blood parameters and estimated energy balance, but cows fed CaSFO lost more body condition. Feeding CaSFO at 19 g/kg of the diet modified the FA composition of milk fat compared with tallow, but did not improve lactational performance of dairy cows. The present findings suggest that supplementation with tallow or CaSFO results in similar lactational performance, with changes in FA profile of milk fat. © 2007 Elsevier B.V. All rights reserved.

Keywords: Calcium salts; Fatty acids; Fish oil; Omega 3; Tallow

1. Introduction

Diets fed to dairy cows are originally low in fatty acid (FA) content, approximately 20 g/kg of the total dry matter (DM). However, supplemental fat sources are utilized in rations for dairy cows as a common method to increase the energy density of the diet to support energy demands for milk synthesis. A wide range of fat sources is available to be fed in the ration of lactating dairy cows, but the adverse effects of some supplements on rumen fermentation and DM intake might offset the benefits of increasing the energy density of the diet (Allen, 2000). In addition, declines in voluntary intake may increase body fat mobilization, decrease plasma glucose, which can compromise health and milk production. The utilization of supplemental fat with a more saturated FA profile, such as tallow, or inert in the rumen, such as calcium salts (CaS), might have less deleterious effect on rumen fermentation than sources rich in unsaturated FA (Pantoja et al., 1994). Digestibility of FA decreased, but modestly, with increased degree of saturation of C18 FA in ruminants (Doreau and Ferlay, 1994). However, when in a triglyceride form, saturated FA tends to be less digestible (Weiss and Wyatt, 2004). Calcium salts have, to some degree, a protective effect against biohydrogenation of unsaturated FA in the rumen (Wu et al., 1991). Therefore, CaS of FA might be a viable technology to reduce the effects of unsaturated FA on the rumen, increase intestinal digestibility of FA, and modify the profile of FA that reach the small intestine. In fact, a recent model to describe ruminal metabolism and intestinal absorption of FA indicated that release of free FA in the rumen is markedly reduced when lipids are in a CaS form (Moate et al., 2004).

Because microbial activity in the rumen resulting in lipolysis and biohydrogenation of lipids containing polyunsaturated FA (PUFA) dramatically reduces the amount of PUFA leaving the rumen (Avila et al., 2000; Doreau and Ferlay, 1994; Wu et al., 1991), methods to reduce the susceptibility of FA to such activity are desirable if the intestinal delivery of specific unsaturated FA is to be achieved in ruminants (Carroll et al., 2006). There are different commercial sources of rumen inert fats including hydrogenated FA and CaS of FA. These fat sources were originally designed to increase the caloric intake of dairy cows with minimal impact on rumen microbial activity; however, more recently, there has been increased interest in improving the flow of PUFA to the lower gut for absorption, such as the n-6 (linoleic acid, C18:2_{n6}) and n-3 (linolenic acid, C18:3_{n3}); eicosapentaenoic acid (EPA), C20:5_{n3}; docosahexaenoic acid (DHA), C22:6_{n3}). Some of these FA may have the potential to improve reproductive performance (Staples et al., 1998; Mattos et al., 2002)

and, if incorporated into milk fat, may improve the nutritional value of milk fat in the human diet (Caggiula and Mustad, 1997).

The current study was designed to evaluate the effect of dietary EPA and DHA supplementation on lactational performance and milk FA composition. In order to increase the supply of dietary EPA and DHA to dairy cows while avoiding their potential negative effects on rumen fermentation, those FA were incorporated into a calcium salt combined with palm oil. Calcium salts are used to make lipids more inert in the rumen and protection of FA from ruminal biohydrogenation in the CaS form is variable, so benefits of feeding fish oil as CaS are expected to be due to both the reduced disturbance of rumen fermentation as compared to free oil, as well as lower biohydrogenation of PUFA. We hypothesized that supplementation with rumen inert fat would benefit rumen fermentation and FA digestibility, which ultimately would result in higher milk production or decreased body fat mobilization. The objective of this study was to evaluate the benefits of replacing rumen active FA from tallow by rumen inert FA from calcium salts of fish oil and palm oils (CaSFO) on lactational performance during the first 145 d postpartum. In addition, the ability of CaSFO in increasing incorporation of PUFA in milk fat, particularly EPA and DHA, was also of interest.

2. Material and methods

2.1. Animals, housing and feeding

Multiparous Holstein cows from a commercial dairy farm in the Central Valley of California were randomly assigned to one of two treatments in a randomized complete block design. Seven-hundred and thirty-eight cows were assigned in experiment 1 (EXP-1) in two replicates per treatment, and 331 cows were assigned to the in experiment 2 (EXP-2) with one replicate per treatment. The EXP-1 was conducted from September 2001 to November 2002, and EXP-2 from May to December 2003. Experiment 2 was basically identical to EXP-1 regarding treatment diets, housing and management practices. Cows were housed in the same free-stall barn, and each pen was identical in size, number of stalls, headlocks, fans, and waterers. Each pen housed the same number of cows at all times.

All cows in both studies were identified with an additional colored ear tag to facilitate identification and daily counts of cows in each study pen. The number of cows was kept constant throughout the study and each pen housed 160 cows (varying from 156 to 160 cows in a given day). All pens were equipped with two rows of fans (2 fans/6 linear meter) facing the stalls, one row located in the back of the stalls, and the row immediately above the stanchions, in the feedbunk. Fans were equipped with high pressure water nozzles and both fans and nozzles were activated once ambient temperature reached 26.0 °C.

Ambient temperature and relative humidity were recorded hourly by data recorders (HOBO® H8 Pro Series Part No. H08-032-08, Onset Computer Corp., Bourne, MA), operated by a computer software program (BoxCar Pro 4.0 Starter Kit, Part No. BCP4.0-ON, Onset Computer Corp., Bourne, MA). Temperature and relative humidity accuracy were within ± 0.15 °C and $\pm 2.2\%$, respectively. Two recorders were placed in each study pen, and were set at a height of 1.8 m from the bedding of the stalls where cows lie down.

The probes recorded data from October 2001 to October 2002 during EXP-1 and, from May to December 2003 in EXP-2. For each 24 h period, average daily mean and maximum temperature and humidity were determined, and the mean and maximum temperature and humidity indexes were calculated. Temperature and humidity index was calculated as follow: dry bulb temperature in °F – [0.55 – (0.55*relative humidity expressed as a decimal)*(temperature – 58)]. Diets were fed to allow for 3 to 5% refusal of the total amount offered, which was estimated once daily when weigh backs were collected and weighed.

Both treatment groups received a similar diet with the only difference being the supplemental fat source, which was included in the total mixed ration (TMR) to supply equal amounts of FA from CaSFO or tallow, in EXP-1 (Table 1) and EXP-2 (Table 2). At calving cows were moved to a common free-stall pen, where all animals in both experiments received a pre-treatment diet during the first 25 DIM consisting of a blend of each fat supplement (200 g/day of FA from tallow + 200 g/day of FA from CaSFO). At 26 DIM cows were moved to specific pens depending on treatment assigned, and treatment diets consisted of 400 g/day of FA from either tallow or CaSFO fed from 26 to 145 days postpartum. The pre-treatment was chosen in order to accommodate the research hypothesis to the regular management of the farm and to introduce cows to both fat sources during the early postpartum period. Also, data from the pre-treatment period were utilized as covariate during statistical analyses. The TMR were fed once daily at 06:00 h, and orts were measure daily for individual pens immediately before feeding.

Table 1
Ingredient composition of experimental diets (Experiment 1)

Ingredients	Treatment ^a	
	CaSFO	Tallow
	g/kg of dry matter	
Alfalfa hay	214	214
Corn silage	254	254
Steam-flaked corn, 360 g/l	155	158
Steam-rolled barley	48	48
Whole cottonseed	90	90
Lignosulfonate soybean meal ^b	43	43
Soybean meal, 470 g/kg CP ^d	31	31
Almond hulls	95	95
Premix ^c	51	51
Tallow	–	18
Calcium salts ^d	19	–

^a CaSFO: calcium salt of palm and fish oils, CP: crude protein.

^b Lignosulfonate treated soybean meal; Amino Plus[®] (Ag Processing, Inc., Emmetsburg, IA).

^c Contains (dry matter basis) 366 g/kg Pro-Lak[®] (blend of marine and animal by-products; H.J. Baker & Bro., Inc., Stamford, CT), 190 g/kg sodium bicarbonate, 87 g/kg potassium carbonate, 96 g/kg Biophos, 73 g/kg calcium carbonate, 45 g/kg magnesium oxide, 35 g/kg magnesium sulfate heptahydrated, 9 g/kg Zinpro 4-Plex[®], 48 g/kg Diamond V[®] yeast culture containing *Saccharomyces cerevisiae*, 11 g/kg Alimet[®], and a mixture of trace minerals and vitamins. Premix contained (dry matter basis): 274 g/kg CP, 57 g/kg Ca, 26 g/kg P, 33 g/kg Mg, 49 g/kg K, 11 g/kg S, 68 g/kg Na, 25 g/kg Cl; and (per kg) 700 mg Zn, 240 mg Cu, 350 mg Mn, 6.2 mg Se, 16.3 mg Co, 15.3 mg I, 83,000 IU of vitamin A, 14,000 IU of vitamin D, and 700 IU of vitamin E.

^d Ener GII-Reproduction Formula (Virtus Nutrition, LLC, Fairlawn, OH).

Table 2
Ingredient composition of experimental diets (Experiment 2)

Ingredients	Treatment ^a	
	CaSFO	Tallow
	g/kg of dry matter	
Alfalfa hay	236	236
Corn silage	185	185
Wheat silage	84	84
Steam-flaked corn, 360 g/l	186	187
Whole cottonseed	67	67
Lignosulfonate soybean meal ^b	39	39
Soybean meal, 470 g/kg CP ^a	47	47
Almond hulls	89	89
Premix ^c	48	48
Tallow	–	18
Calcium salts ^d	19	–

^a CaSFO: calcium salt of fish and palm oils, CP: crude protein.

^b Lignosulfonate treated soybean meal; Amino Plus[®] (Ag Processing, Inc., Emmetsburg, IA).

^c Contains (dry matter basis) 163 g/kg Sea-Lac[®] (Menhaden fish meal, Omega Protein, Houston, TX), 246 g/kg blood meal, 225 g/kg sodium bicarbonate, 26 g/kg potassium carbonate, 58 g/kg Biophos, 112 g/kg calcium carbonate, 83 g/kg magnesium oxide, 12 g/kg Zinpro 4-Plex[®], 52 g/kg Diamond V[®] yeast culture containing *Saccharomyces cerevisiae*, 21 g/kg Alimet[®], and a mixture of trace minerals and vitamins. Premix contained (dry matter basis): 274 g/kg CP, 57 g/kg Ca, 26 g/kg P, 33 g/kg Mg, 49 g/kg K, 11 g/kg S, 68 g/kg Na, 25 g/kg Cl; and (per kg) 700 mg Zn, 240 mg Cu, 350 mg Mn, 6.0 mg Se, 16.3 mg Co, 13.2 mg I, 85,000 IU of vitamin A, 20,000 IU of vitamin D, and 800 IU of vitamin E.

^d Ener GII-Reproduction Formula (Virtus Nutrition, LLC, Fairlawn, OH).

Diets were sampled weekly, dried at 55 °C for 48 h and ground in a Wiley mill (Arthur H. Thomas CO., Philadelphia, PA) to pass a 2-mm screen. Samples were then composited, ground in a cyclone mill (Udy Co., Fort Collins, CO) to pass a 1 mm screen and analyzed for DM (AOAC, 2000, Method # 925.40), ash (AOAC, 2000, Method # 923.03), crude protein (CP; AOAC, 2000, Method # 984.13) and minerals. Acid detergent fiber (ADF) and lignin (sa) were determined according to AOAC (2000; Method # 973.18) and sulfuric acid was utilized to solubilize the cellulose. Neutral detergent fiber (aNDF) was assayed utilizing a heat stable amylase according to Van Soest et al. (1991) and without sodium sulfite. Acid detergent fiber and aNDF are expressed including the residual ash. The mineral content of diets was determined at the Dairyland Laboratory (Arcadia, WI) using an inductively coupled plasma mass spectrometer (Thermo Jarrell-Ash, Franklin, MA). Fatty acids from feed samples were determined as methyl esters, which were obtained by acidic methylation of FA according to (Sukhija and Palmquist, 1988) utilizing heptane as solvent. Gas chromatography conditions and peak identifications were performed as described for milk FA. Body condition from all cows in EXP-1 was scored (Ferguson et al., 1994) by the same person at 4, 70, 98, and 123 ± 3 DIM.

2.2. Lactational performance and milk fatty acid profile

Cows were milked three times daily and milk yields were recorded for individual cows once monthly during the official California DHIA test in EXP-1, and twice a month during

EXP-2. Milk samples were analyzed for somatic cell count (SCC; AOAC, 2000, Method # 975.16), fat, and true protein concentrations by mid-infrared spectroscopy (Foss 303 Milk-O-Scan®; Foss Foods, Inc.; Eden Prairie, MN) according to AOAC (2000, Method # 972.16) at the DHIA Laboratory in Hanford, CA. All cows received 500 mg of exogenous bovine somatotropin (Posilac®, Monsanto Co., St. Louis, MO) every 14 days, starting at 63 ± 3 days postpartum.

Milk samples (a.m./p.m. composited) from a subgroup of 37 cows in EXP-1, 18 fed tallow and 19 CaSFO, at 55 ± 4 DIM were collected, kept frozen at -25°C , and later analyzed for total fat as described previously (AOAC, 2000, Method # 972.16). Fatty acid profile was determined by separation of methyl esters with a Hewlett Packard 5890 gas chromatograph equipped with a 100 m capillary column (0.32 mm, 0.20 μm film thickness; Supelco 2560, Supelco Inc., Bellefonte, PA) utilizing hydrogen as the carrier gas, as described in details by DePeters et al. (2001). Briefly, milk fat was extracted according to methodology described by Erickson and Dunkley (1964), and methyl esters were obtained by alkaline methylation utilizing 100 μl of 3 M potassium hydroxide solution. The retention time for each specific FA was determined by utilizing an external standard composed of monoacids and mixed chain triglycerides (Nu-chek Prep, Inc., Elysian, MN). The concentrations of FA in the sample were obtained by back calculating FA concentration based on the peak area and the known concentration of FA in the standard.

2.3. Blood sampling and biochemical analyses

Blood (7 ml) was sampled from the median coccygeal artery or vein from a subgroup of 20 cows (9 tallow and 11 CaSFO) at 0, 15, 30, 45, 60, 90, 120, 150, and 180 min after feeding (minute 0) when cows were at 63 ± 2 DIM during EXP-1 to measure the concentrations of glucose and nonesterified FA (NEFA) in plasma. Evacuated tubes containing 17.55 mg of K_2 EDTA (Vacutainer®, Becton Dickinson, Franklin Lakes, NJ) were utilized and plasma was obtained from blood after centrifugation at $2000 \times g$ for 15 min in a refrigerated centrifuge at 5°C . Plasma was then stored at -25°C until analyzed for glucose and NEFA. Glucose concentrations in plasma were analyzed based on the glucose oxidase reaction utilizing a biochemical analyzer YSI 2700-S BioChem (Yellow Springs Instrument Co. Inc., Ohio, USA). Plasma NEFA concentrations were determined using a colorimetric kit (Wako Chemicals GmbH, Neuss, Germany) utilizing a protocol described by Johnson and Peters (1993).

2.4. Chemical analyses of *n*-alkanes and DM intake estimation

A subgroup of 35 cows from EXP-1 was dosed with a slow release capsule (MCM alkane, code n. 60421, batch n.0-600, CAPTEC, NZ, <http://www.capttec.info>) containing the even-chained pair of *n*-alkanes, C_{32} (*n*-dotriacontane) and C_{36} (*n*-hexatriacontane). The manufacturer guidelines indicate that the capsule delivers approximately 350 mg/day of each even-chained *n*-alkane for approximately 23 days, estimated by *in vivo* disappearance rate in rumen-fistulated cows. In order to obtain a representative estimate of DM intake during the study, 19 cows fed CaSFO and 16 fed tallow were dosed at 43 ± 6 and again at 93 ± 6 DIM, from which 16 and 14 cows contributed with data for both sampling periods for

CaSFO and tallow, respectively. Fecal sampling started 8 days after capsule administration, at 51 ± 6 (early lactation) and 101 ± 6 DIM (mid-lactation), once daily, after the morning milking, for a period of 7 consecutive days. Fecal samples were dried in a forced air oven at 45°C for approximately 48 h. Dried samples ($n = 7/\text{cow}$) were then composited for each cow within each period and DM at 105°C was determined from the composited samples. Feed was sampled daily ($\sim 1 \text{ kg}/\text{treatment}/\text{day}$) during the fecal sampling periods. Feed samples were dried and composited as described for fecal samples. Alkane extraction from feces and feeds were performed as described by Mayes et al. (1986) with the following modifications. Feed (1.5 g) and feces (1.0 g) were subjected to direct saponification in an ethanolic KOH solution (1M). A solution of C_{22} and C_{34} diluted in heptane (150 ppm) were utilized as an internal standard. After liquid extraction, the extracts were filtered through a silica gel column (5 ml bed; 70–210 mesh, Sigma–Aldrich, St. Louis, MO 63178, USA), eluted with $5 + 5 + 5 \text{ ml}$ of *n*-heptane, concentrated to $800 \mu\text{l}$, transferred to a scintillation vial, gently evaporated, and frozen at -25°C until analyzed. The extracts were re-diluted in $250 \mu\text{l}$ of heptane and an aliquot of $1 \mu\text{l}$ was injected into a Hewlett Packard 5890 Capillary Gas Chromatograph fitted with a J & W DB-1 capillary column, with a split vent flow rate of $55 \text{ ml}/\text{min}$. Initial temperature of the column was held for 0.5 min after injection at 280°C and increased at a rate of $0.5^\circ\text{C}/\text{min}$ until a final temperature of 325°C was reached, resulting in a total run time of 10 min. Injector and detector temperatures were both set at 325°C , with carrier gas (hydrogen) head pressure of 20 p.s.i., resulting in a liner velocity of $55 \text{ cm}/\text{s}$.

Dry matter intake for individual cows was estimated during early and mid-lactation according to calculations developed by Mayes et al. (1986) and modified by Vulnich et al. (1991), based on the ratio of natural and dosed *n*-alkanes, as follow:

$$\text{Intake} = \frac{D_{32} * (\text{Fc}_{31}/\text{Fc}_{32})}{\text{Fe}_{31} - ((\text{Fc}_{31}/\text{Fc}_{32}) * \text{Fe}_{32})} \quad (1)$$

where D_{32} is the amount of dosed *n*-alkane ($350 \text{ mg}/\text{day}$ of C_{32}), Fc_{31} and Fc_{32} are the fecal concentrations (mg/kg DM) of C_{31} and C_{32} , and Fe_{31} and Fe_{32} are the TMR concentrations (mg/kg DM) of C_{31} and C_{32} , respectively.

Ratio of C_{31} and C_{32} (natural:dosed) was utilized rather than C_{33} : C_{32} as concentrations of C_{33} in the diet were much lower than C_{31} , 10.99 and $92.89 \text{ mg}/\text{kg}$ of DM, respectively. Total tract apparent digestibility of DM was estimated by the relationship of *n*-alkane (C_{33}) concentration in the TMR and feces, utilizing the equation below (Unal and Garnsworthy, 1999):

$$\text{Digestibility} = \frac{(\text{C}_{33\text{f}}/\text{rr}) - \text{C}_{33\text{d}}}{(\text{C}_{33\text{f}}/\text{rr})} \quad (2)$$

where $\text{C}_{33\text{f}}$ is the concentration of *n*-alkane in feces (mg/kg DM), $\text{C}_{33\text{d}}$ is concentration in the diet (mg/kg DM) and rr is the 0.88 fecal recovery rate of *n*-alkanes reported by Unal and Garnsworthy (1999).

2.5. Experimental design and statistical analyses

Cows in both experiments were blocked based on parity, month of expected calving, and 305-days milk production during the previous lactation. Within each block cows were randomly assigned to one of the two treatment diets. Data from 44 cows in EXP-1 were not included because they did not remain in the treatment groups for the entire study period or did not have measurements of yields of milk and components for at least 3 months. The remaining 694 cows were included in the data analyses. In EXP-2, 331 multiparous cows provided data for the statistical analyses.

Data were tested for normal distribution of the residues by the PROC UNIVARIATE procedure of SAS release 8.2 statistical software package (SAS, 2001). The data were assumed to have residues normally distributed when the Shapiro-Wilk statistic was equal or greater than 0.90, or when the shape of the stem-and-leaf plot resembled the Gaussian curve, otherwise the data were submitted to mathematical transformation (Strum et al., 2000).

Production data were analyzed utilizing the production traits from the first 25 days postpartum as covariate, as both treatments received the same diet during this period. Mathematical model for EXP-1 included the following effects: treatment, BCS at calving, time, pen replicate, and interactions. A similar model was utilized to analyze data from EXP-2, however the effects of BCS at calving and pen replicate were not included.

All continuous data were analyzed as repeated measures (Littell et al., 1998) using the MIXED procedure of SAS (2001). The Akaike's information criterion was utilized to identify the best covariance structure for each dependent variable analyzed (Littell et al., 1998).

Differences with $P \leq 0.05$ were considered significant and $P > 0.05$ but $P \leq 0.10$ were considered as a tendency. All data shown are least square means.

3. Results

3.1. Experiment 1

The nutrient composition of the diets was similar in CP, aNDF, fat, and minerals (Table 3). However, because of the experimental design with use of fat sources differing in FA profile, the FA composition of the TMR differed. The ration containing CaSFO contained greater concentration of EPA, DHA, and palmitic acid, whereas the ration with tallow contained more oleic acid (Table 3). However, the concentrations of EPA and DHA in the TMR of cows fed CaSFO were lesser than expected based on the composition of the CaS and their inclusion into the diet. Cows fed tallow consumed small quantities of EPA and DHA because the diet contained some fish meal as part of a commercial rumen undegradable-protein supplement.

Temperature and humidity evaluated as daily mean or maximum of pens in both treatments were similar ($P > 0.25$) throughout the study. Cows were exposed to thermal stress during the months of May to September, when the temperature and humidity index in all pens in both treatments was above 72. The average daily maximum temperature and humidity index for those months were 76.3 and 76.1 for CaSFO and tallow pens, respectively ($P = 0.79$).

Table 3

Chemical composition (g/kg of dry matter \pm S.D.) of experimental diets and fatty acid profile for the supplemental fats (Experiment 1)

Parameter (g/kg of dry matter)	Treatment ^a	
	CaSFO	Tallow
NE _L , MJ/kg of dry matter ^b	6.78	6.74
Organic matter	916 \pm 4	919 \pm 3
Crude protein	173 \pm 3	173 \pm 7
RUP ^c	70.2	70.2
Non-fiber carbohydrate ^c	373 \pm 19	382 \pm 31
aNeutral detergent fiber	315 \pm 14	311 \pm 18
Acid detergent fiber	227 \pm 34	213 \pm 16
Lignin (sa)	43 \pm 4	48 \pm 4
Fat	56 \pm 8	53 \pm 11
Calcium	10.5 \pm 0.6	9.6 \pm 0.5
Phosphorus	4.7 \pm 0.1	4.7 \pm 0.2
Magnesium	3.7 \pm 0.3	3.8 \pm 0.3
Potassium	1.71 \pm 0.04	1.65 \pm 0.13
Sodium	4.2 \pm 0.1	4.1 \pm 0.1
Chloride	4.3 \pm 0.4	4.1 \pm 0.4
Fatty acid, g/100 g of fatty acids ^d		
C14:0	1.3 \pm 0.07	1.3 \pm 0.02
C16:0	30.2 \pm 1.18	23.9 \pm 0.18
C16:1	0.9 \pm 0.04	1.2 \pm 0.09
C18:0	3.9 \pm 0.17	7.0 \pm 0.01
C18:1 <i>cis</i> 9 + <i>cis</i> 10	22.5 \pm 0.50	22.6 \pm 0.52
C18:2 <i>cis</i> 9, <i>cis</i> 12, n-6	34.1 \pm 2.57	35.7 \pm 1.48
C18:3, n-3	3.5 \pm 0.44	4.0 \pm 0.46
C20:5, n-3 (EPA) ^e	0.19 \pm 0.01	0.06 \pm 0.01
C22:6, n-3 (DHA) ^e	0.18 \pm 0.02	0.05 \pm 0.01
Fatty acid profile from the supplemental fats ^d		
Fatty acids		g/100 g of fatty acids
C14:0	2.4 \pm 0.09	2.8 \pm 0.13
C16:0	40.9 \pm 0.40	26.1 \pm 0.56
C16:1	2.0 \pm 0.14	3.2 \pm 0.21
C18:0	4.2 \pm 0.34	19.8 \pm 0.48
C18:1 <i>cis</i> 9 + <i>cis</i> 10	30.2 \pm 0.36	36.3 \pm 0.50
C18:2 <i>cis</i> 9, <i>cis</i> 12, n-6	7.5 \pm 0.23	5.6 \pm 0.32
C18:3, n-3	0.55 \pm 0.01	0.70 \pm 0.07
C20:5, n-3 (EPA) ^e	2.3 \pm 0.11	Not detected
C22:6, n-3 (DHA) ^e	2.5 \pm 0.10	Not detected
Saturated FA ^e	46.6 \pm 0.47	50.9 \pm 0.67
Unsaturated FA ^e	53.5 \pm 0.37	49.1 \pm 0.35
PUFA ^e	12.8 \pm 0.56	6.3 \pm 0.48

^a CaSFO: calcium salts of fish and palm oils.

^b Net energy for lactation calculated according to NRC (2001) and adjusted for 26 kg/day of DM intake.

^c Non-fiber carbohydrate, g/kg = 100 – (aNeutral detergent fiber, g/kg + crude protein, g/kg + fat, g/kg + ash, g/kg); RUP, g/kg = ruminally undegradable protein according to NRC (2001) adjusted for 26 kg of DM intake.

^d Average of 6 and 3 samples, for CaSFO and tallow, respectively. Dry matter (g/kg) and total fatty acids (g/kg of DM) were, respectively, 960.4 \pm 0.6 and 807 \pm 4.3 for CaSFO, and 970 \pm 1.4 and 999 for tallow.

^e EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; PUFA: polyunsaturated fatty acids; FA: fatty acids.

Table 4

Lactational performance of dairy cows fed calcium salts of fish and palm oils (CaSFO) or tallow (Experiment 1)

Parameter	Treatment		S.E.M.	P-value
	CaSFO	Tallow		
Cows, <i>n</i>	346	351	–	–
Group dry matter intake, kg/day	25.8	25.0	1.01	0.86
Milk, kg/day	48.6	48.8	0.30	0.64
Milk fat				
g/kg	37.8	38.0	0.3	0.50
kg/day	1.84	1.86	0.02	0.57
Milk true protein				
g/kg	27.5	28.2	0.1	0.01
kg/d	1.34	1.37	0.01	0.02
Linear somatic cell count score	3.09	3.26	0.09	0.23
Somatic cell count, ×1000/ml	385	478	–	–

3.1.1. Milk yield, composition and fatty acid profile

Yields of milk and milk fat were not affected by source of FA in the diet (Table 4). However, concentration of true protein ($P<0.01$) and yield of true protein ($P<0.02$) in milk were reduced when cows received CaSFO compared with tallow. Somatic cell count was unaffected by type of supplemental fat.

Feeding CaSFO increased ($P<0.01$) the concentration of total n6 FA and PUFA in milk fat (Table 5). Despite the small amount of fish oil fed in this experiment, approximately 3 to 4 g/kg of diet DM, the concentration of DHA in milk fat from cows fed CaSFO was 71% greater ($P<0.01$), and of EPA slightly increased ($P<0.01$) compared with milk fat from cows fed tallow. Linoleic acid was also increased in milk fat from cows fed CaSFO. Although concentrations of PUFA were significantly increased in milk fat, changes were not large enough to improve the thrombogenic and atherogenic indexes for milk fat from cows fed CaSFO. The concentration of C18:1 *trans* 9, C18:1 *trans* 11, and conjugated linoleic acid C18:2 *cis* 9 *trans* 11 were all increased ($P<0.01$) in milk fat of cows fed CaSFO compared with tallow, which indicates that that FA from CaSFO had greater influence on rumen biohydrogenation than FA from tallow.

3.1.2. Dry matter intake, blood biochemistry and body condition

Group DM intake (Table 4) was in agreement with DM intake estimated by the digesta marker using *n*-alkanes (Table 6), and type of supplemental fat had no significant impact ($P=0.39$). Digestibility of DM was greater ($P=0.03$) for cows fed CaSFO than tallow, however yields of milk, milk components, and estimated NE_L concentration of TMR were similar between diets. Daily energy output in milk was similar ($P=0.57$) between the two diets and averaged 146.5 MJ of NE_L /cow. Similarly, energy provided by BCS change was similar ($P=0.12$) between treatments. The lack of differences in energy intake and energy output by cows resulted in similar, but negative, mean net energy balance from 50 to 100 DIM (Table 6).

Table 5

Milk fatty acid (FA) profile for cows^a fed calcium salts of fish and palm oils (CaSFO) or tallow (Experiment 1)

Fatty acids	Treatment		S.E.M.	P-value
	CaSFO	Tallow		
Cows, <i>n</i>	19	18	–	–
	g/100 g of FA			
C4:0	5.62	5.42	0.14	0.33
C6:0	2.42	2.57	0.04	<0.01
C8:0	1.25	1.37	0.03	<0.01
C10:0	2.57	2.88	0.09	0.03
C12:0	2.76	3.09	0.11	0.04
C13:0	0.09	0.10	<0.01	0.28
C14:0	9.68	10.43	0.21	0.02
C14:1 <i>cis</i>	0.72	0.82	0.04	0.13
C15:0	0.86	0.94	0.03	0.12
C16:0	31.75	29.94	0.55	0.03
C16:1 <i>trans</i>	0.38	0.35	0.02	0.21
C16:1 <i>cis</i>	1.60	1.59	0.07	0.93
C17:0	0.56	0.68	0.01	<0.01
C18:0	11.98	13.08	0.40	0.06
C18:1 <i>trans</i> 9*	0.38	0.34	0.02	<0.01
C18:1 <i>trans</i> 11	2.15	1.46	0.10	<0.01
C18:1 <i>cis</i> 9 + <i>cis</i> 10	20.86	21.05	0.54	0.8
C18:2 <i>cis</i> 9, <i>cis</i> 12, n-6	2.89	2.63	0.06	<0.01
C18:3, n-3	0.38	0.38	<0.01	0.9
C18:2 <i>cis</i> 9, <i>trans</i> 11 (CLA) ^b	0.76	0.53	0.04	<0.01
C20:4, n-6*	0.16	0.18	<0.01	0.31
C20:5, n-3 (EPA) ^b	0.043	0.038	<0.01	<0.01
C22:6, n-3 (DHA) ^{b,*}	0.024	0.014	<0.01	<0.01
Unsaturated long chain fatty acids	27.66	26.62	0.60	0.22
n-3 fatty acids	0.45	0.44	<0.01	0.19
n-6 fatty acids	3.05	2.81	0.06	<0.01
Index of atherogenicity ^c	2.68	2.76	0.10	0.5
Index of thrombogenicity ^d	3.68	3.71	0.11	0.8

^a Milk sampled (a.m./p.m. composited) from 37 cows at 64 ± 4 days in milk (P=0.80).

^b CLA: conjugated linoleic acid; EPA: eicosapentaenoic acid; and DHA: docosahexaenoic acid.

^c Index of atherogenicity = [(C12:0 + (C14:0*4) + C16:0)] / [(n-3 FA*3) + n-6 FA + (C18:1, *cis* 9 & *cis* 10) + C16:1, *cis* + C14:1 *cis*].

^d Index of thrombogenicity = (C14:0 + C16:0 + C18:0) / [(n-3 FA*3) + n-6 FA + (0.5*C18:1, *cis* 9 & *cis* 10) + (0.5*(C16:1, *cis* + C14:1 *cis*) + (0.5*n-6 FA) + (3*n-3 FA) + (n-3 FA/n-6 FA)]. Both indexes were calculated according to Ulbricht and Southgate (1991).

* Errors not normally distributed, P-values based on analysis of transformed data (1/sqrt).

Postprandial changes in NEFA concentrations (Fig. 1) were not affected by dietary treatments during the first 3 h after feeding, but plasma concentrations of glucose were greater (P<0.04) for cows fed CaSFO than tallow. The BCS was smaller (P<0.02) for cows fed CaSFO than tallow, and this difference was observed for the entire study period (Fig. 2). However, the difference in losses of BCS occurred during the first 70 days of lactation as determined by the interaction (P<0.02) between treatment and time on BCS, and the parallelism of changes after 70 days postpartum.

Table 6
Production traits, dry matter (DM) intake, and DM digestibility in a subgroup of 35 cows (Experiment 1)

Parameter	Treatment		S.E.M.	P-value
	CaSFO	Tallow		
Cows, <i>n</i>	19	16	–	–
DM intake, kg/day ^a	26.6	27.7	0.89	0.39
DM digestibility, g/kg of DM ^b	598	575	7.0	0.03
Milk, kg/day	49.6	49.9	1.5	0.88
Milk fat, g/kg	40	37	1.1	0.13
Milk protein, g/kg	27.1	27.7	0.4	0.28
Milk energy output, MJ NE _L ^c	149	144	5.0	0.57
Energy balance (MJ NE _L /day), 50 to 100 DIM ^d	–7.45	–2.55	4.4	0.58
Energy from BCS loss, MJ NE _L ^e	–343	–101	109	0.12
Estimated NE _L of diets, MJ/kg ^f	7.0	6.7	–	–

^a Estimated by the ratio of dietary (C31) and dosed (C32) *n*-alkanes at the beginning (52.5 ± 3 DIM) and end of the trial (100.8 ± 3 days in milk) according to Eq. (1) described in the materials and methods.

^b Estimated using the concentration of *n*-alkane (C33) in the diet and feces according to the equation 2 described in the materials and methods.

^c Milk energy, MJ of net energy of lactation = $4.187 * [\text{milk yield} * ((0.929 * \text{fat g/kg}) + (0.563 * \text{true protein g/kg}) + 0.192)]$ (NRC, 2001).

^d Energy balance = (DM intake * estimated dietary NE_L) – (milk energy + maintenance). DIM: days in milk.

^e The loss of one unit of body condition score (BCS) provided 1746 and 1671 MJ of NE_L, for BCS loss from calving to 70 and from 70 to 100 DIM, respectively (NRC, 2001).

^f Net energy of lactation = [(milk energy output + maintenance energy requirements) – (energy from body condition change)/DM intake]. Maintenance was calculated to be 44 MJ/day based on average body weight of 670 kg at the first week of lactation; NRC (2001).

3.2. Experiment 2

As expected, and similar to EXP-1, the chemical composition of the diets was similar, but with distinct FA profiles (Table 7). Because of the differences in FA profile of the supplemental fat sources, the diet containing CaSFO had greater concentration of EPA, DHA, linoleic acid, and palmitic acid, whereas tallow contained more stearic and oleic acids (Table 7). The concentrations of EPA and DHA in the TMR were greater in EXP-2 than in EXP-1, although the FA profile of the CaSFO were similar in both experiments.

Cows were exposed to thermal stress from the months of June to September, when the temperature and humidity index in treatment pens reached values above 72, which characterizes exposure to thermal stress for lactating dairy cows (West, 2003). Throughout the study, mean temperature, humidity, temperature and humidity index measured at the level of the cow were similar between study pens and they were, respectively, 21.1 °C, 60.4%, and 66.5 for CaSFO, and 21.4 °C, 60.6%, and 66.8 for tallow. These data indicate that cows in both treatments were exposed to similar environmental conditions of temperature and humidity.

3.2.1. Milk yield and composition

Results of EXP-2 resembled those of EXP-1. Group DM intakes were 24.5 and 24.2 kg/day for cows fed CaSFO and tallow. Yields of milk and milk fat, and concen-

Table 7

Chemical composition (g/kg of dry matter \pm S.D.) of experimental diets and fatty acid profile for the supplemental fats (Experiment 2)

Parameter (g/kg of dry matter)	Treatment ^a	
	CaSFO	Tallow
NE _L (MJ/kg of dry matter) ^b	6.87	6.82
Organic matter	901 \pm 2	914 \pm 2
Crude protein	171 \pm 1	173 \pm 2
RUP ^c	71.3	72.1
Non-fiber carbohydrate ^d	389 \pm 2	392 \pm 2
aNeutral detergent fiber	302 \pm 0.2	311 \pm 2
Acid detergent fiber	219 \pm 28	221 \pm 21
Lignin (sa)	43 \pm 4	48 \pm 4
Fat	56 \pm 1	59 \pm 1
Calcium	9.1 \pm 0.4	8.6 \pm 0.3
Phosphorous	4.2 \pm 0.2	4.4 \pm 0.2
Magnesium	3.6 \pm 0.2	3.5 \pm 0.3
Potassium	15.4 \pm 0.5	15.0 \pm 0.5
Sodium	4.2 \pm 0.2	4.0 \pm 0.2
Chloride	3.2 \pm 0.1	3.3 \pm 0.2
Fatty acid, g/100 g of fatty acids		
C14:0	1.8 \pm 0.07	1.6 \pm 0.10
C16:0	31.8 \pm 0.11	24.2 \pm 0.45
C16:1	1.6 \pm 0.19	1.9 \pm 0.18
C18:0	3.2 \pm 0.28	19.2 \pm 0.31
C18:1 <i>cis</i> 9 + <i>cis</i> 10	18.2 \pm 0.28	24.3 \pm 0.55
C18:2 <i>cis</i> 9, <i>cis</i> 12, n-6	33.1 \pm 0.34	22.4 \pm 0.58
C18:3, n-3	0.4 \pm 0.04	0.6 \pm 0.08
C20:5, n-3 (EPA) ^e	0.8 \pm 0.10	0.1 \pm 0.01
C22:6, n-3 (DHA) ^e	0.9 \pm 0.12	0.1 \pm 0.01
Fatty acid profile from the supplemental fats ^d		
Fatty acids		g/100 g of fatty acids
C14:0	2.5 \pm 0.09	2.9 \pm 0.13
C16:0	40.1 \pm 0.50	26.0 \pm 0.43
C16:1	2.2 \pm 0.21	3.4 \pm 0.20
C18:0	4.1 \pm 0.31	21.6 \pm 0.52
C18:1 <i>cis</i> 9 + <i>cis</i> 10	30.2 \pm 0.36	36.1 \pm 0.51
C18:2 <i>cis</i> 9, <i>cis</i> 12, n-6	7.2 \pm 0.29	3.9 \pm 0.36
C18:3, n-3	0.51 \pm 0.02	0.66 \pm 0.04
C20:5, n-3 (EPA) ^e	2.3 \pm 0.14	Not detected
C22:6, n-3 (DHA) ^e	2.6 \pm 0.13	Not detected
Saturated FA ^f	45.8 \pm 0.64	50.4 \pm 0.74
Unsaturated FA ^f	54.2 \pm 0.55	49.6 \pm 0.81
PUFA ^f	12.6 \pm 0.62	4.6 \pm 0.76

^a CaSFO: calcium salts of fish and palm oils.

^b Net energy for lactation calculated according to NRC (2001) and adjusted for 26 kg/day of DM intake.

^c Non-fiber carbohydrate, g/kg = 100 – (aNeutral detergent fiber, g/kg + crude protein, g/kg + fat, g/kg + ash, g/kg); RUP, g/kg = ruminally undegradable protein according to NRC (2001) adjusted for 26 kg of DM intake.

^d Average of three samples for CaSFO and tallow. Dry matter (g/kg) and fat (g/kg of DM), respectively, was 971 \pm 1.3 and 811 \pm 5.8 for CaSFO, and 959 \pm 2.2 and 989 \pm 2.2 for tallow.

^e EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

^f PUFA: polyunsaturated fatty acids; FA: fatty acids.

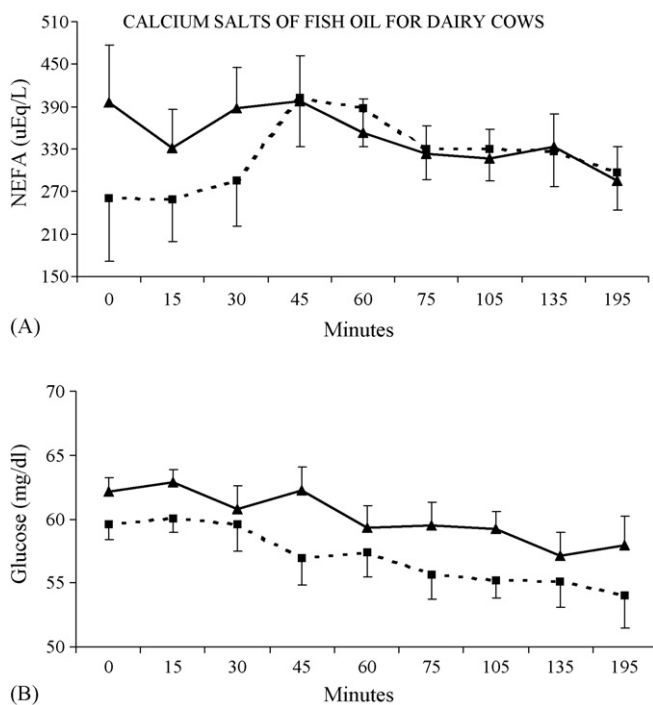


Fig. 1. Postprandial changes in concentrations of nonesterified fatty acids (NEFA; A, $\mu\text{Eq/l}$) and glucose (B, mg/dl) in plasma from a subset of 40 cows in experiment 1, 20 fed calcium salt of fish and palm oils (CaSFO; solid line; \blacktriangle) and 20 tallow (dashed line; \blacksquare) during the first 3 h post feeding (minute 0). Plasma concentrations of NEFA did not differ ($P>0.80$), but glucose was greater ($P<0.04$) for cows fed CaSFO than those fed tallow. Pooled S.E.M. was 51.2 and 0.98 for NEFA and glucose, respectively.

tration of fat in milk were similar for cows fed CaSFO and tallow (Table 8). However, feeding CaSFO reduced the concentrations of true protein ($P=0.05$), lactose ($P=0.02$), and of solids-not-fat ($P=0.06$) in milk. The changes in milk true protein concentration resulted in a decrease ($P=0.04$) in milk protein yield for cows fed CaSFO compared to those fed tallow. No differences in linear somatic cell score were observed according to type of supplemental fat fed.

4. Discussion

Because tallow had a high degree of saturation (509 ± 0.4 g/kg saturated FA; Table 3), it was expected that feeding tallow would cause no negative effects on rumen fermentation or, at most, minor modifications. However, its ability to increase the incorporation of PUFA into milk fat is limited by the low content of PUFA and lack of protection against biohydrogenation. In contrast, CaSFO had more than twice as much PUFA (12.7 versus 5.45 g/100 g of FA), which were potentially protected against biohydrogenation, particularly

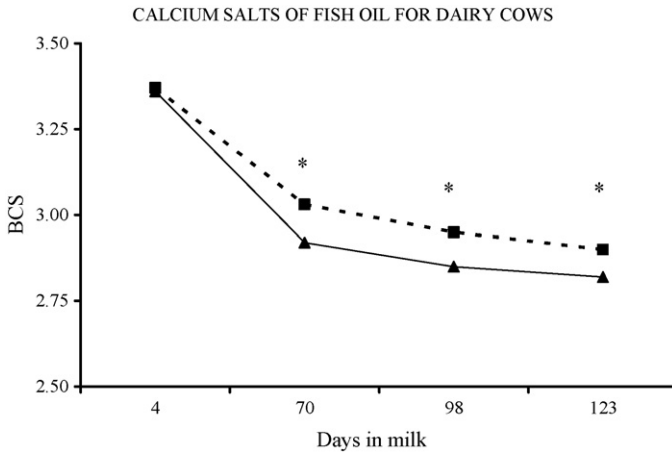


Fig. 2. Body condition score (BCS) during the first 123 days in milk for cows fed calcium salt of fish and palm oils (solid line; ▲) or tallow (dashed line; ■) in experiment 1. Pooled S.E.M. = 0.016. Effect of treatment ($P < 0.01$) and treatment by time interaction ($P < 0.02$) were observed. Asterisks indicate differences between treatments at each time point ($P < 0.05$).

linoleic acid, EPA and DHA. These characteristics would qualify CaSFO as an appropriate dietary fat supplement to replace tallow and alleviate any negative effect of PUFA on rumen fermentation and improve the incorporation of PUFA in animal products.

Yields of milk and milk fat were not affected by the source of dietary FA in both experiments reported. In both experiments, however, feeding CaSFO reduced yields and concentrations of milk true protein. In EXP-2, not only milk true protein, but solids-not-fat were also suppressed for cows fed the CaSFO compared with tallow.

Table 8

Lactational performance of dairy cows fed calcium salts of fish and palm oils (CaSFO) or tallow (Experiment 2)

Parameter	Treatment		S.E.M.	P-value
	CaSFO	Tallow		
Cows, <i>n</i>	165	166	–	–
Milk, kg/day	44.9	45.5	0.44	0.28
Milk fat				
g/kg	35.4	35.6	0.3	0.60
kg/day	1.59	1.63	0.02	0.17
Milk true protein				
g/kg	27.3	27.6	0.1	0.05
kg/day	1.22	1.26	0.01	0.04
Milk lactose, g/kg	48.1	48.7	0.4	0.02
Milk solids-not-fat, g/kg	83.8	84.5	0.8	0.06
Linear somatic cell count score	3.37	3.42	0.11	0.75
Somatic cell count, $\times 1000/\text{ml}$	319.6	381.2	–	–

Calcium salts of palm oil increased milk production in some studies (Scott et al., 1995; Harrison et al., 1995), although these positive results have not always been consistent throughout the lactation (Garcia-Bojalil et al., 1998). Staples et al. (1998) reviewed the effects of feeding fats on reproduction in dairy cows, but also summarized their effects on milk yield and DM intake. Addition of CaS of palm oil increased milk yield by 2.4 kg/day, in spite of a reduction in DM intake of 0.5 kg/day compared to a non-supplemented group. Reductions in intake caused by CaS were also summarized by Allen (2000). Similarly, adding tallow to the diet increased milk yield by 0.9 kg/day, with a small reduction in DM intake (Staples et al., 1998). Supplementing lactating cows with CaS of palm oil seems to benefit production when CaS either replace unsaturated fat sources or increase the FA content of the diet. However, no comparisons in the literature were found between CaS and tallow for dairy cows. Feeding hydrogenated palm oil triglycerides (34 g/kg of DM), a highly saturated fat source (>900 g/kg of saturated FA), decreased milk production compared to cows fed CaS of palm oil FA, but 4% fat-corrected milk production was not affected (Weiss and Wyatt, 2004). This decrease in milk production was observed in spite of similar DM intakes and was attributed to the lower digestibility and consequently lower digestible energy content of the saturated palm oil triglyceride diet compared to diet containing CaS of palm oil FA.

Response to tallow supplementation seems to be dependent upon type of forage fed (Onetti et al., 2004; Ruppert et al., 2003), with negative effects observed in diets predominantly based on corn silage. Most of the negative effects observed are related to DM intake, rumen fermentation parameters, and concentration of fat in milk, but not necessarily yields of milk (Onetti et al., 2004; Ruppert et al., 2003). Indeed, lower rumen pH and higher content of total C18-*trans* FA in milk fat indicated that rumen fermentation was negatively affected in cows fed corn silage either as the main or the only forage source (Onetti et al., 2002; Onetti et al., 2004; Ruppert et al., 2003). These data suggest that interaction of fat and fiber sources is an important determinant of response to fat sources, and inclusion of alfalfa hay into a diet based heavily upon corn silage improves production responses to fat sources that are not considered inert in the rumen. In some studies, when tallow was fed at 20 g/kg of the diet DM, type of forage had marginal effects on response to supplemental fats (Onetti et al., 2002), but milk fat contained a greater proportion of C18-*trans* FA. In both experiments reported herein, alfalfa hay comprised at least 210 g/kg of the diet DM, which probably minimized any possible negative effects of tallow on productive performance. In fact, DM intake either measured as a group with two replicates per treatment or for individual cows using *n*-alkanes did not differ between cows fed tallow or CaSFO.

Recently, a preliminary report by Castaneda-Gutierrez et al. (2005) demonstrated that when CaS of fish oil was fed through the rumen fistula, the negative effects of fish oil on DM intake and lactation performance were minimized compared to feeding the free oil. In that study, infusion of fish oil in the rumen markedly suppressed DM intake and milk fat yield compared with abomasal infusion of fish oil or feeding of CaSFO. These data indicate that CaS of fish oil minimized the negative effects of fish oil on rumen fermentation. When fish oil was supplemented unprotected, 145 g/day (Castaneda-Gutierrez et al., 2005), 206 g/day (Petit et al., 2002) or 290 to 612 g/day (Donovan et al., 2000) milk fat content and yield were depressed. Donovan et al. (2000) demonstrated a linear negative effect of fish oil (added at 0, 10, 20 and 30 g/kg of diet DM) on milk fat content and yield. Fish oil supplementation

greater than 10 g/kg of DM (290 g/day) decreased DM intake and milk production, although cows fed 10 g/kg fish oil produced more milk than cows not supplemented with fish oil. Moreover, increasing unsaturation of unprotected FA decreased DM intake (Pantoja et al., 1994) and compromised rumen fermentation. Therefore, two factors likely explain the lack of negative effects of fish oil in the current study on DM intake and yields of milk and milk fat. First, fish oil was fed as a CaS, which likely minimized its effects on the rumen microflora (Castaneda-Gutierrez et al., 2005). Secondly, the amount of fish oil consumed by cows in experiments 1 and 2 was approximately 80 g/day (3.2 g/kg of the diet DM), which is substantially less than that demonstrated to affect performance (Donovan et al., 2000).

Milk protein content and yield were negatively affected by feeding CaSFO. Supplementation with fish oil previously decreased milk protein content (Ahnadi et al., 2002; Petit et al., 2002) or milk protein yield. In some studies, feeding fish oil did not affect protein content of milk, but reduction in protein yield was a consequence of the depression in feed intake and milk production in response to feeding fish oil (Donovan et al., 2000; Shingfield et al., 2003). Even when small amounts of EPA and DHA from fish oil were fed, for example approximately 10 g/cow/day, they decreased milk protein content in relation to diets that contained similar amounts of crude fat but without any fish oil (Petit et al., 2002), suggesting a direct effect of FA from fish oil on protein synthesis or amino acid uptake in the mammary gland. Indeed, when glutaraldehyde-protected and unprotected fish oil were supplemented at rates of 15 to 37 g/kg of dietary DM to dairy cows, mRNA for β -casein was significantly decreased for the higher levels of fish oil compared to the low level (15 g/kg of DM), and protein content was decreased in cows fed the high level of protected fish oil (Ahnadi et al., 2002). Furthermore, Drackley et al. (1992) observed a linear negative effect between degree of unsaturation for abomasally-infused oil and yield of both, casein N and true protein N in milk. The similar degree of unsaturation from the supplemental fat sources and from the milk FA profile in the present studies suggests that FA in CaSFO, possibly EPA and DHA might be implicated in the lower milk protein content and yield observed when cows were fed CaSFO compared with tallow.

Donovan et al. (2000) fed increasing amounts of fish oil and observed a linear effect on concentration of EPA in milk fat, from 0.22 to 0.40 g/100 g of FA, but only moderate changes in concentration of DHA. Secretion of EPA and DHA in milk depends on both, absorption in the small intestine and uptake by the mammary gland. In fact, when equal amounts of EPA and DHA were infused into the rumen as CaSFO or as fish oil, concentration of EPA and DHA into milk fat did not improve by feeding fish oil in a CaS form (Castaneda-Gutierrez et al., 2005). Therefore, the changes in concentrations of EPA and DHA in milk fat were likely due to the greater intake of these FA in cows fed CaSFO than tallow, as opposed to extensive protection against microbial biohydrogenation.

Ulbricht and Southgate (1991) developed two indices alternatively to the traditional ratio of PUFA:saturated FA as an attempt to classify food according to its likelihood to predispose humans to coronary heart disease, the thrombogenic and atherogenic indices. Even though EPA, DHA and linoleic acid were increased in milk fat from cows fed CaSFO, and saturated FA (C12, C14 and C18) was decreased, these changes were not sufficient to improve the thrombogenic and atherogenic indices of milk fat from cows fed CaSFO (Table 5). Our results suggest that changes in milk FA profile to improve the healthfulness of milk fat for human consumption would require either greater intake of EPA and DHA as CaSFO

or greater degree of protection against rumen biohydrogenation (Castaneda-Gutierrez et al., 2005). Feeding CaSFO increased the content of certain *trans* FA in milk fat including, C18:1 *trans* 9, C18:1 *trans* 11, and conjugated linoleic acid C18:2 *cis* 9 *trans* 11, which suggests that rumen production of these FA was increased. *Trans* FA are the product of incomplete biohydrogenation of unsaturated FA in the rumen, such as oleic and linoleic acids, however the content of these FA in dietary fat was similar across the two diets, suggesting that a portion of the fish oil was released in the rumen as free FA and altered the biohydrogenation process. Indeed, supplementation with fish oil increased more than three fold the concentration of *trans* FA in milk fat when cows were fed diets with similar linoleic acid content (Whitlock et al., 2002).

Concentrations of NEFA in plasma can reflect the degree of adipose tissue mobilization, but might also be affected by the supply of FA to the small intestine (Drackley et al., 1992). Blood concentration of NEFA reflects the balance between release of FA from adipose tissue and lipoproteins, and utilization of NEFA by tissues, such as the mammary gland. Given the similar production of milk and milk fat between treatments, it would be reasonable to assume that utilization of NEFA by the mammary gland was similar between groups. Small changes in plasma NEFA are observed with fat supplementation, but, in early lactation, most of the changes in NEFA concentrations are the result of changes in energy status and adipose tissue mobilization (Drackley, 1999). In the current study, the lack of change in plasma NEFA concentrations (Fig. 1) was probably due to similar DM intakes and energy output in milk. Although cows fed CaSFO experienced greater losses of BCS in the first 70 days postpartum, calculated energy balance was similar for both treatments. In spite of the greater DM digestibility and similar DM intake, feeding CaSFO did not improve energy status of early lactation dairy cows.

During the postprandial period, cows fed CaSFO had greater concentrations of glucose, which can be related to plasma insulin concentrations. When unsaturated FA in a CaS form replaced hydrogenated prilled FA, plasma insulin concentration was decreased by 27%, whereas DM intake declined by only 0.7 kg (Harvatine and Allen, 2005). In addition, supplementation with fish oil decreased the insulin response to glucose of perfused pancreatic islets in rats (Pighin et al., 2003). These findings might explain the increased concentration of glucose in cows fed CaSFO. It is unlikely that the greater concentration of glucose in plasma for cows fed CaSFO was the result of a better energy status, as this cannot be substantiated by DM intake, plasma NEFA, BCS change, and calculated energy balance.

5. Conclusion

Replacement of FA from tallow by FA from CaSFO had no effect on yields of milk and milk fat, but reduced concentrations and yields of true protein and solids-not-fat in milk. Feeding CaSFO altered the composition of FA in milk fat and increased the concentrations of EPA and DHA by 13.1 and 71.4%, respectively. However, changes in FA composition were not large enough to improve the atherogenic and thrombogenic indexes of milk fat. Calcium salts of fish and palm oils improved DM digestibility, but did not alter concentrations of blood metabolites and resulted in a slight increase in losses of BCS early postpartum. The present study does not support the current feeding recommendation of replacing rumen

available fat by rumen inert fat containing polyunsaturated FA in order to improve lactational performance, at least when tallow is the source of rumen available FA in the diet and alfalfa comprises a reasonable portion of the dietary forage.

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